

The Gregarines of *Glycera siphonostoma*.

By

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With Plate 18.

THE large Polychaet, *Glycera siphonostoma* D. Ch. (sometimes placed in a separate genus *Rhynchobolus*), is at times infested with numerous gregarines, both intestinal and cœlomic. On this account it is somewhat difficult to differentiate the stages in the life-history of anyone of them. Out of fifty-two specimens examined at Naples in March and April, 1914, twelve (I to IX, XII, XIV, and XXVI) were infected with a species of *Gonospora* which presented some interesting points. The affinities of this form with previously established species will be discussed later. It does not agree in detail with the published account of any, notwithstanding the fact that Léger (11) has already recorded *Gonospora sparsa* as occurring in some undetermined species of *Glycera* at Belle Isle.

A specimen of *Gl. siphonostoma* infected with *Gonospora* can generally be detected in the living, for, through its body-wall, both the large attached trophozoites and free cysts can be distinguished. The latter move backwards and forwards suspended in the cœlomic fluid, as the host expands and contracts. The large trophozoites are attached to the thick muscular pharynx from the region of the jaws to the intestine (Pl. 18, fig. 1). The numbers and size of the individual parasites vary very much in different specimens. Sometimes only one or two occur, but in the case illustrated (VI) they

were numerous towards the posterior half of the pharynx. In another (IX) a fringe of trophozoites was found just in front of the jaws. The length of the single trophozoites varies from about 1 mm. to 4 or 5 mm. in length. Narrow at the attached end, they gradually widen out, and then taper to a blunt point. The smallest specimen shown in Pl. 18, fig. 1, is at *e*. The small projections at *f* were thought to be possibly young forms, but on cutting sections of this part they were discovered to be only the remains of the attached ends of associates which had become free. The nucleus is spherical and near the widest part of each individual; it contains generally four or five caryosomes (Plate 18, fig. 4). Each of the parasites is covered with a layer of the host's cœlomic epithelium, thicker in some parts than in others. It seems clear, as will be further explained later, that the parasites of a certain length, having reached the cœlom, attach themselves to the pharynx. Thereupon they penetrate a little into the host's tissue, and the peritoneum, greatly increasing in the neighbourhood, rapidly grows round the parasite, forming a layer in contact with, but not attached to, the exterior cuticle of the gregarine. This covering of host's cells does not keep pace with the growth of the trophozoite, which consequently has to become bent on itself to some extent, especially at its narrow attached end (Plate 18, fig. 4).

The trophozoites evidently revolve to a certain extent about their points of attachment, and in this way the free extremities of two forms may come together as at *a* and *b* (Pl. 18, fig. 1), when association proceeds to take place. The layer of host tissue is eliminated from between the contiguous extremities, and the end of one associate projects into the end of the other, which consequently becomes cup-shaped, and thus union of the two associates is made secure (Pl. 18, figs. 2 and 3). This dove-tail arrangement reminds one very forcibly of the cup and ball structure described by Huxley (10) in *Ganymedes*, and I would suggest that in this gregarine also the distinctive ends, which the author states he could not always find, were probably only temporary forms taken on by the parasites at

the beginning of association. That is at any rate the case here. Before association all the parasites have regularly tapering ends. A somewhat similar mode of association of two or several individuals was described by Caullery and Mesnil (3) in *Gonospora longissima*, where they state that the extremity of one associate sometimes forces itself into the other, invaginating it "en doigt de gant." They compare this with the similar phenomenon in the *Didymophyes* of Stein. In this polycystid gregarine, however, it is the satellite which attaches itself to the primate of a syzygy in this way (17, Taf. IX, fig. 40), and therefore necessarily the attachment is by opposite ends. In the *Gonospora* from *Gl. siphonostoma* it is union of similar ends that is affected in this secure way. Following this association the pair continue to rotate, and since the proximal ends are still attached to the pharynx they become much convoluted and shortened (Pl. 18, figs. 2 and 3). It would have been interesting to see what would have happened during shortening of the attached ends in the case of the associates in Pl. 18, fig. 1, marked *a* and *b*, which, it will be seen, have become intertwined. In Pl. 18, fig. 2, is shown a case of attempted triple association. Evidently after the firm union of two specimens a third has become attached, and is seeking to come into closer relation, though as yet separated by much host tissue. In normal cases the associated ends gradually enlarge and become rounded off, forming a spherical cyst, into which is drawn up most of the protoplasm from the attached ends. A little, however, may be left with its covering of host tissue outside the cyst, as in *Kalpidorhynchus*¹ (7). The cuticle of the trophozoite may be seen to be closely ribbed by examining the ball end of an associate. After formation of the cyst the cup and ball arrangement disappears and the thin partition between the gametocytes becomes straightened out, the remaining cuticle thickening slightly to form the cyst wall. Meanwhile the covering provided by the host forms a thick wall round the associates, and its proximal parts shorten and

¹ The correct generic name of this parasite is discussed on p. 213.

thicken, but continue to attach the spherical cyst to the pharynx for some time. There is no organic connection between parasite and host cyst. The latter may easily be removed at any time (Pl. 18, fig. 3).

During the changes recorded above nuclear division has been proceeding in each gametocyte. The stage showing the first nuclear division has not been seen. It would take place in a couple at a stage between those represented in Pl. 18, fig. 1, by *a* and *b* on the one hand, and *c* on the other. For in the pair of associates *a* and *b* there were only the trophozoite nuclei in each, while in *c* there were several nuclei which were still dividing. Here it may be mentioned that although all these nuclei appear similar, there is at this early stage a much greater number in one associate than in the other. A similar observation is recorded by Cunningham (7, p. 205) in the case of his *Kalpidorhynchus arenicolæ*, namely, that one gametocyte has fewer nuclei than the other. This difference is here at any rate only transitory, for at the stage represented by *d* (Pl. 18, fig. 1) there seems to be the same number of nuclei in each gametocyte; and there is still no difference to be distinguished in the size or appearance of the nuclei of the two gametocytes. However, Brasil (1) stated that in *G. varia* the difference in appearance of the nuclei of the two gametocytes only appeared distinctly on the passage of the nuclei to the surface formed by their convolutions (1, p. 31). Unfortunately these later stages are wanting among my cysts. Both the formation and fusion of the gametes appear to be gone through rapidly at the time when the cyst breaks away from the pharynx and becomes free in the cœlom. The youngest of these cysts obtained free in the cœlom still had processes at either side where the host's cyst had broken off from the short stumps *f* (Pl. 18, fig. 1) left attached to the pharynx. This cyst contained, besides some residual protoplasm, numerous young spores each with an undivided syncaryon (Pl. 18, fig. 5). Two older cysts containing ripe spores had become completely spherical, showing no signs of having been attached. Round

these the host cells formed a colourless layer of uniform thickness.

SPORES.

The spore is provided with a very characteristic wall composed of endospore and exospore, and having a funnel at one end (Pl. 18, fig. 6). The exospore is thick and transparent, and is supported by processes running through it from the endospore. Similar, though longer, processes run up and support the sides of the funnel. The appearance of the latter is evidently what has given rise to the general statement that in *Gonospora* the spore is terminated by a crown of spines—"couronne de fines pointes hyalines," Léger (11, p. 156) and Brasil (1, p. 20). When unstained, or washed out, the endospore and its processes are refringent, but they can be shown up much more clearly by overstaining with iron-hæmatoxylin. The endospore measures about $10\ \mu$ by $8\ \mu$. The exospore is very delicate and easily overlooked unless the illumination is very good. The processes of the endospore enlarge slightly towards their outer ends, sometimes appearing to have globular extremities. They might almost be minute canals, out of which a sticky fluid was oozing. At any rate, granules in the neighbourhood adhere easily to them. Ripe spores contain eight sporozoites, which escape through the funnel. The thickness of the fully-formed spore-coat makes it difficult for reagents to penetrate it and stain the contents (Pl. 18, fig. 6).

Only on the one occasion, cited above, were cysts with ripe spores found free in the cavity, and those had evidently only just become detached from the pharynx. In all other cases such cysts were embedded in brown masses composed of host phagocytes. Their brown colour was due to granules and other waste products removed from the cœlom. These masses were sometimes very large, one, in specimen VI, was 9 mm. long by 1 mm. wide, and contained embedded in it about a dozen cysts with ripe spores. More usually they measured less than 2 or 3 mm. in any direction; sometimes

they contained small trophozoites in addition to spores, at other times necrotic Nematodes and their eggs, and very often old broken setæ. They give, in fact, evidence of the very effective way in which the host is destroying its parasites together with other useless matter. The ultimate destruction of these masses in *Glycera siphonostoma* has been described by Goodrich (9, p. 456), as taking place in the nephridial sacs. Much has been written also on general phagocytic action of the leucocytes of annelids, and destruction of parasites by them has been described by Siedlicki (15), Cuenot (5 and 6), Caullery and Mesnil (2), and others. I am unable, however, to find any recorded instance of phagocytes having to attack a layer of their own host's tissue in order to reach their quarry. In *Gl. siphonostoma* they appear to attack first any free foreign bodies, and it is only after dealing effectively with these that an onslaught is made on attached forms. The attacking phagocytes can easily be distinguished from the covering cells of ordinary cœlomic epithelium by their larger size, branching character, and brown colour due to enclosed granules. In one specimen (IX) a brown mass free in the cœlom was found to contain four trophozoites and four cysts, all in a necrotic condition. The only other parasites were three or four small trophozoites attached quite close to the jaws. Two of these were being held together laterally by a mass of phagocytes with the usual brown granules. These two trophozoites, in spite of their covering of colourless host cells, were evidently in process of being detached from the pharynx and destined to ultimate destruction by phagocytes. This is the only sign of anything like lateral association that I have been able to observe in this form, and of course here it is really nothing of the kind, but merely a case of two small trophozoites being conveniently destroyed at the same time by the host. Léger, however, described lateral association in *G. sparsa*, a form found by him in *Glycera* sp. This makes me hesitate to connect this parasite from *Gl. siphonostoma* with that from other Glycerids until such time as it may be possible to

compare the living forms. To prevent confusion, therefore, it is perhaps well to refer to the form under consideration as *G. glyceræ*.

SYSTEMATIC POSITION OF GONOSPORA GLYCERÆ N.SP.

The genus *Gonospora* was established by Schneider (14, p. 597) in 1875 to include a species found by him in "*Audouinia lamarkii* at Roscoff and also in Terebellids." The trophozoite of this form is described as elongated, broader at one end than the other and the spores as oval, without processes. This would appear to be not unlike Léger's *G. varia* from *Audouinia* (see below). Unfortunately Schneider called this species *G. terebellæ* Köll., although he stated immediately after that he dared not affirm that his species was the same as Kölliker's *Gregarina* (*Monocystis*) *terebellæ*. If, however, the description and figure of the latter given by Kölliker (10a, fig. 6) be studied it will be clear that this form should be included in the genus *Selenidium*. This fact has already been pointed out by Dogiel (8) in his interesting work on the gregarines.

On the whole, then, it seems reasonably clear that the type species on which Schneider founded the genus *Gonospora* was the one subsequently named *G. varia* by Léger in 1892. The chief known characteristics of this and the other two species hitherto described (all apparently forms free in the cœlom) may be summed up as follows:

(1) *G. varia*, Léger, from the cœlom of *Audouinia* (11, p. 157), further described by Brasil (1, p. 21). Trophozoites may be 2 mm. long; association terminal; gametes anisogamous; spores oval, 18 to 21 μ long.

(2) *G. sparsa*, Léger, from the cœlom of *Phyllodoce* and *Glycera* sp. (11). Trophozoites elongated, attaining a length of 1 mm.; association lateral; spores 10 μ long, nearly spherical with "couronne de pointes hyalines."

(3) *G. longissima* C. and M. (2 and 3) from cœlom of *Dodecaceria*. Trophozoites very large; association terminal and intimate; spore apparently oval (not clearly figured).

In none of the above cases, I venture to think, has the spore been studied at a sufficiently high magnification to reveal its true characteristics. Dogiel (8) has recorded the existence of a funnel, and it seems possible that there may also be found a more complicated coat than has been described. In the meanwhile it may be pointed out that the spore of the species from *Gl. siphonostoma* in size and shape much resembles *G. sparsa*; but for the present its distinguishing characteristics may be summarised as follows:

(4) *G. glyceræ* n. sp. from the cœlom of *Glyceria siphonostoma* D. Ch. Trophozoites attached to pharynx during the greater part of their life and covered with a layer of the host's cœlomic epithelium; association terminal and made secure by a dovetail arrangement; spores with a refringent endospore which gives off processes supporting the thick transparent exospore with its funnel.

In addition to *Gonospora* several other gregarines have been found in *Gl. siphonostoma* which have not been shown to have any connection with it. A few observations, however, on these may be of assistance to anyone who should have a chance of obtaining this polychæte at Naples and wish to continue the study of the parasites. I have not been able to obtain any living specimen since leaving Naples, and equally unsuccessful have been efforts to obtain from the British coasts *Gl. gigantea*, *Quatrefages*, which McIntosh (12) maintains is the same species.

(1) *Cystobia intestinalis* Ssok. was obtained in three specimens (namely, IV, XXII, and LII). This parasite was described by Ssokoloff (16) from specimens occurring in a preserved intestine of a *Gl. siphonostoma* (*Rhynchobolus*) which he received from Naples. In this case the whole of the sporogony is described as taking place in the intestinal wall. In my specimen of *Gl. siphonostoma* with the best infection, while there are numerous trophozoites single or associated in the intestinal wall (Pl. 18, fig. 7), there are also very many free. Among these the only pair of associates found in which nuclear division has started is free in the

lumen. Unfortunately no later stages are represented at all, so that I am unable to confirm the account given by Ssokoloff from his somewhat inadequate material. This author does not refer to the peculiar dense meshwork of the ectoplasm which contains small deeply staining granules, although it can be distinguished in one of his micro-photographs.

In his classification (p. 227) Ssokoloff has omitted *C. minchinii*, established by Woodcock (18) as a species occurring in *Cucumaria*, but has included the badly-defined *C. schneideri* Ming., which was stated by Cuenot (4, p. 4), so early as 1892, to be identical with *C. holothuriæ*. He has also included Cunningham's *Kalpidorhynchus arenicolæ* among *Cystobia*, as advocated by Dogiel (8).¹

(2) Another gregarine occurring abundantly in at least three specimens (II, VIII, and XXIV) of *Gl. siphono-*

¹ That this last parasite is a monocystid Gregarine is quite clear from an examination of specimens which can easily be found in *Arenicola ecaudata* at Plymouth. Since it does not undergo neogamy (i. e. precocious association, Woodcock (18)), it should be included in the genus *Gonospora* rather than in *Cystobia*. The same is probably true of the parasite under consideration called *Cystobia intestinalis* by Ssokoloff, but its life history requires confirmation.

The characteristics of the spores of Gregarines are much more important, from a systematic point of view, than such details in their life history as the manner of association (whether terminal or lateral), or the time at which it takes place (whether neogamous or normal). I cannot, therefore, agree with Woodcock as to the necessity of the genera *Diplodina* (Woodcock, 1906), and *Cystobia* (Ming., 1891), in which, with one exception mentioned below, the spores are practically identical with those of *Gonospora*. The occurrence of neogamy, though of much interest, is not of systematic value. Woodcock himself points out that this condition is developed to a varying extent even in a single species, viz. *Diplodina irregularis* (18, pp. 60 and 63), and also that the condition occurs to some extent in other genera such as *Diplocystis*, *Zygocystis* (pp. 62 and 63). This being the case it seems preferable to place the two species of *Diplodina*, viz. *D. (Cystobia) irregularis* and *D. (Cystobia) minchinii*, in the genus *Gonospora*, and *Cystobia holothuriæ* (the spore of which is provided with a short flattened tail as well as a funnel) in the genus *Lithocystis* (13a). Woodcock (18, p. 60) fully realised that such were their relationships.

stoma is a cœlomic form always seen in pairs (Pl. 18, fig. 8). These are generally very much smaller than the *Gonospora* trophozoites attached to the pharynx, and have no apparent relationship with them. Specimens measured varied between .2 and 1.6 mm. in length and were never more than 1 mm. wide. They were attached to the body-wall, retractor muscles, or intestinal wall, never to the pharynx. The two individuals of a pair are held together and attached to the host by a homogeneous secretion (Pl. 18, fig. 8, *m*), which extends between them for about one third of their length from the attached extremities. The cuticle is thin and the protoplasm finely granular. The large, oval nucleus varies in position, but is generally nearer the distal end; it is limited by a well-marked membrane and sometimes contains a couple of caryosomes.

Presumably this form has no more connection with *C. intestinalis* than with *Gonospora glyceræ*, and I merely make these few observations in case they may be of use in subsequent investigations.

(3) In two specimens (II and IV) free or attached monocystid gregarines were found in the intestine, especially towards the posterior end. There was nothing especially characteristic about these, and it is unlikely that they should have any connection with the previously described forms.

(4) In one specimen (III) small active gregarines were found in the anterior region of the intestine. These were probably the sporozoites of one of the above forms, although no spores were seen.

SUMMARY.

(1) There appear to be four different gregarines parasitic in *Gl. siphonostoma*, including at least one species of *Gonospora*.

(2) *Gonospora glyceræ* n. sp. is surrounded throughout the greater part of its existence by a layer of host epithelium. Association is made secure by means of a dovetail arrangement. The spores, under a high magnification, reveal a more

complicated structure than has previously been described in the genus.

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EXPLANATION OF PLATE 18,

Illustrating Mrs. Helen L. M. Pixell-Goodrich's paper on
“The Gregarines of *Glycera siphonostoma*.”

(All preparations, unless otherwise stated, were stained with iron-hæmatoxylin and drawn with the aid of a camera lucida.)

Fig. 1.—Posterior region of pharynx of *Glycera siphonostoma* showing attachment of single and associated Gonospora. *a, b, c, d*, pairs of associates in order of development; *e*, young trophozoite; *f*, remains of attached ends left by liberated cysts. Drawn after fixation in corrosive acetic mixture. $\times 8$.

Fig. 2.—Optical section of distal ends of two associates, *a* and *b*, showing intimate union between them, and a third specimen, *c*, attempting to effect multiple association. Stained iron-hæmatoxylin. $\times 23$.

Fig. 3.—A pair of associates slightly compressed, after removal of envelope of host cells. Drawn from the living. $\times 23$.

Fig. 4.—Optical section of a single trophozoite, after being detached from pharynx, showing small nucleus with several caryosomes. *h*, Covering of host's cells; *p*, proximal end bent on itself. $\times 75$.

Fig. 5.—Optical section of young spore, with characteristic coat just formed. The nucleus (syncaryon) starting to divide, but much overstained. $\times 2000$.

Fig. 6.—Ripe spore showing characteristic endospore with processes, faintly staining exospore, and funnel supported by slightly longer processes. $\times 2000$.

Fig. 7.—*Cystobia intestinalis* Ssok. Section through trophozoite at base of intestinal cells. *n*, Remains of hypertrophied nucleus of host cell. *ec*, Dense ectoplasm with deeply staining granules. $\times 500$.

Fig. 8.—A pair of cœlomic gregarines held together by a structureless membrane (*m*), extending to nearly half their length from the attached ends. $\times 250$.